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Nucleotide sequence of the EcoRI fragment from pLJ3 bearing two tandem lacUV5 promoters

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The plasmid pLJ3, a derivative of pMB9, contains a small EcoRI fragment bearing two tandem lacUV5 promoters (1). This EcoRI fragment was constructed in such a way that it consists of four segments: an EcoRI-AluI segment containing the first lacUV5 promoter, an AluI-HaeIII segment, a HaeIII-AluI segment, and an AluI-EcoRI segment containing the second lacUV5 promoter. All four segments were obtained from AluI fragments from λplac5. The four AluI fragments were engineered via a complex series of steps to yield the EcoRI fragment of pLJ3 (1). pLJ3 has frequently been utilized as a source of the lacUV5 promoter for a variety of purposes (2-7). The nucleotide sequence of this EcoRI fragment was determined by the dideoxy method.

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EcoRI                                                                +1
GAATTCTCAC TCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATAATGT GTGGAATTGT
                            PvuI/AluI                  HaeIII
GAGCGGATAA CAATTTCACA CAGGAAACAG CTGATTGCCC TTCACCGCCT GGCCTCCGTT GAGCCATCTG
                                  SphI           AluI
GATCGGCAGC GTTGTCTTCA TCAACCGGAA CGAGCATGCC GGAGAGCAGC TCACTCATTA GGCACCCCAG
                                        +1
GCTTTACACT TTATGCTTCC GGCTCGTATA ATGTGTGGAA TTGTGAGCGG ATAACAATTT CACACAGGAA
   EcoRI
ACAGAATTC
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Key restriction sites are shown above the DNA sequence, and +1 marks the startpoint of transcription for the two lacUV5 promoters. The 22 bp AluI-HaeIII segment is from the lacI gene (bp 1009 to 1030 of the Genbank sequence); the 67 bp HaeIII-AluI segment is presumably from a previously unsequenced portion of the E. coli chromosome carried on λplac5. In addition to being a useful source of the lacUV5 promoter, we have found that this EcoRI fragment functions as an efficient transcription terminator. Others have reported transcription termination associated with the lac promoter (8-11).

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